Red cell volume expansion at altitude: a meta-analysis and Monte Carlo simulation

Rasmussen, Peter ; Siebenmann, Christoph ; Díaz, Víctor ; Lundby, Carsten

Abstract: INTRODUCTION: Altitude acclimatization is associated with a rapid increase in hematocrit. The time course and the contribution of the red cell volume expansion are not clear. The purpose of the present meta-analysis was to explore how much altitude exposure is required to induce polycythemia in healthy lowlanders. METHODS: A systematic review was performed of 66 published articles (including 447 volunteers) identified through literature search. We performed a mixed-model random-effects meta-analysis and a Monte Carlo simulation on the extracted data. RESULTS: The following results were obtained in this study: 1) the red cell volume expansion for a given duration of exposure is dependent on altitude ($P < 0.0001$), that is, that the increase in red cell volume was accelerated at higher altitudes; and 2) the extent of the erythropoietic response depends on the initial red cell volume ($P < 0.0001$). It seems that exposure time must exceed 2 wk at an altitude of more than 4000 m to exert a statistically significant effect. At lower altitudes, longer exposure times are needed with altitudes lower than 3000 m not yielding an increase within 4 wk. CONCLUSIONS: Red cell volume response to hypoxia is generally slow, although it accelerates with increasing altitude. This, in combination with a dependency on initial red cell volume, suggests that, for example, athletes may need to spend more time at altitude to see an effect on red cell volume than commonly recommended.

DOI: https://doi.org/10.1249/MSS.0b013e31829047e5

Red cell volume expansion at altitude: a meta-analysis and Monte-Carlo simulation.

Peter Rasmussen[1,2,3†], Christoph Siebenmann[1,2†], Víctor Díaz[1,4,5†], and Carsten Lundby[1,2].

1. Zurich Center of Integrative Human Physiology, Institute of Physiology, Zurich, Switzerland
2. Institute of Physiology, University of Zurich, Zurich, Switzerland
3. Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark
4. Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland
5. Department of Health and Human Performance, Faculty of Sport Science – INEF, Technical University of Madrid, Madrid, Spain

[†] Contributed equally to the manuscript.

Running title: Red cell expansion at altitude

This study was supported by a grant from the Danish National Research Council (10-094110) and the Zurich Center of Integrative Human Physiology. All authors wrote the paper, approved the final version and declare no conflicts of interest.

Corresponding author:
Peter Rasmussen
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich
Switzerland
+41791024002
+41446356814
peter@prec.dk

Abstract

Introduction: Altitude exposure and acclimatization is associated with a rapid increase in hematocrit. The time course and contribution of the red cell volume expansion is not clear. The purpose of the present meta-analysis is to explore how much altitude exposure is required to induce polycythemia in healthy lowlanders.

Methods: A systematic review of 66 published papers (including 447 volunteers) identified through literature search. We performed mixed-model random-effects meta-analysis and Monte-Carlo simulation on the extracted data.

Results: 1) that red cell volume expansion for a given duration of exposure is dependent on altitude ($P<0.0001$) i.e. that the increase in red cell volume was accelerated at higher altitudes, and 2) that the extent of the erythropoietic response depends on the initial red cell volume ($P<0.0001$). It appears that exposure time must exceed 2 weeks at an altitude of more than 4,000 m to exert a statistically significant effect. At lower altitudes, longer exposure times are needed with altitudes below 3,000 m not yielding an increase within 4 weeks.

Conclusion: Red cell volume response to hypoxia is generally slow although it accelerates with altitude. This, in combination with a dependency on initial red cell volume, suggests that e.g. athletes may need to spend more time at altitude to see an effect on red cell volume than commonly recommended.

Keywords: altitude training, hemoglobin mass, hypoxia, systematic review.

[Paragraph Number 1.]

Introduction

Hypoxia leads to stabilization of the Hypoxia Inducible Factor-2 (HIF-2) system in the renal peritubular cells which subsequently binds to the hypoxic response element on the (Epo) gene and induces synthesis and release of Epo [11]. As a results, plasma Epo concentrations start to increase after approximately two hours of hypoxic exposure [5]. As exposure continues a zenith is reached after 3-4 days, where after plasma Epo concentration gradually decreases to stabilize slightly above sea level values [3,19,25].

[Paragraph Number 2.]

The primary function of EPO is to stimulate the maturation of reticulocytes in the bone marrow. Furthermore, permanent residence at altitude is associated with an augmented total red cell volume (RCV) [25] which has led to the persisting paradigm that extended hypoxic exposure induces polycythemia in lowlanders. Nevertheless, the Epo response to hypoxia does not necessarily translate into an elevated RCV [1,23]. In fact, the initial increase in hematocrit at altitude is entirely the result of a plasma volume contraction that occurs within the first hours/days of exposure [22]. In contrast, the time course of RCV expansion and the dependency on the degree of hypoxia remain largely unknown. This is related to the inconvenience of exposing a sufficient subject number to different altitudes for a prolonged time. To overcome this problem we conducted a meta-analysis of published experiments and explored the duration and degree of altitude exposure required to induce polycythemia in healthy low-landers.

[Paragraph Number 3.]

Methods

Search strategy

This meta-analysis is based on papers retrieved from the web-based data bases PubMed and Web of Science (See selection process and reference list, Supplemental Digital Content). Up to January 2012, we introduced the following key words and Boolean connectors.

(altitude OR hypoxia) AND acclimatization AND (haemoglobin mass OR hemoglobin mass OR haematocrit OR hematocrit OR red blood cell mass OR red blood cell volume OR blood volume OR plasma volume)

The search was refined by applying the limits *Humans* and *All adult: +19 years* in PubMed and searching within the subject areas of *Physiology, Respiratory system, Cardiovascular system cardiology, Sport Sciences* and *Hematology* in the Web of Science. This initial search generated a group of 6,775 references that were transferred to an EndNote (Thomson Reuters, New York, USA) data base.

[Paragraph Number 4.]

In EndNote, duplicate references were removed. From the remaining references *in vitro* or animal studies, reviews and conference proceedings were eliminated. The abstracts of the 446 remaining papers were then independently evaluated by two researchers and papers not fulfilling the eligibility criteria (see below) were removed. Disagreements were resolved by involving the rest of the research team. At the end of this process we selected 53 papers.

Subsequently, we built a reference map for each paper and obtained the references cited in or citing the initial 53 papers. This step retrieved a new group of 3185 papers (1503 backward and 1682 forward references) and the same process detailed above and in figure 1 was repeated. Finally, 66 papers were selected to be included in the meta-analysis, see Supplemental Digital Content.

[Paragraph Number 5.]

Eligibility criteria

Any study on healthy humans reporting data before and after a period of exposure to hypoxia (normobaric or hypobaric, continuous or intermittent) for blood compartments (plasma volume, RCV or blood volume) or total haemoglobin mass was eligible. Only investigations using direct methods of measurement (Evans blue, CO-rebreathing, radioiodinated albumin or red-brilliant dilution) were accepted. Studies on high altitude natives, reviews, conference proceedings, editorials and letters to the editor were rejected. Studies in which the experimental procedures included any intervention susceptible of affecting any blood compartment, other than physical activity, were eliminated as well.

[Paragraph Number 6.]

Data extraction

The data were transferred into an Excel file by two researchers working independently, and subsequently compared to avoid error. When data were extracted from figures, the mean between the values obtained by the two researchers was taken.

When the degree of altitude was not constant a time-weighted average was calculated.

$$\text{Average altitude} = \frac{\sum_{i=1}^{n} \text{Dur.}_i \times \text{Alt.}_i}{\text{Total duration}}$$

$$= \frac{\text{Dur.}_1 \times \text{Alt.}_1 + \text{Dur.}_2 \times \text{Alt.}_2 + \cdots + \text{Dur.}_n \times \text{Alt.}_n}{\text{Total duration}}$$

where Dur.i and Alt.i are the duration and altitude at step "i". So for, e.g., a stepwise accent to Mt. Everest [20] the average altitude is derived as [1 day x 3,800 m + 5 days x 4,500 m + 5 days x 5,000 m + 2 days x 5,500 + ... etc]/36 days = 5,720 m. If only SEMs were reported, variance was calculated from sample size and SD, otherwise variance was calculated from SD alone.

[Paragraph Number 7.]

Statistical analysis

Mixed-model random-effects meta-regression was performed (SAS 9.2, SAS institute) on log response ratios [10]. The log response ratio was calculated as the logarithm to the change in RCV i.e. the post value divided by the pre/control value. We chose RCV as our effect parameter due to the linear relationship with haemoglobin mass, whereas hematocrit and hemoglobin concentration was excluded for their dependency on plasma volume. If red cell volume was missing it was derived from the linear relationship between hemoglobin mass and RCV. This relationship was obtained from 13 studies (n=28) where both hemoglobin mass and RCV were reported (RCV = hemoglobin mass * 2.899, $P < 0.0001$). In general, none of the included studies reported confidence intervals or standard deviations on the change in RCV, rather SD (or SEM) was reported on the pre and post values separately. Variance was therefore corrected as proposed for cross-over trials [4]. P-values below 0.05 were considered statistically significant.

[Paragraph Number 8.]

Analysis and results

Overall subject characteristics are reported in **Table 1**. In total this analysis reports data from 447 volunteers of which 376 were male and 71 female. RCV increases versus exposure time and altitude are shown in **Fig. 1**. On average, the RCV increase per week was 49±240ml/week.

[Paragraph Number 9.]

Heterogeneity

Due to the inclusion of papers spanning different methodologies (plasma dye dilution, carbon monoxide re-breathing or radioactive albumin labeling), altitudes (from 1,300 to 6,000 meters above sea-level), exposure times (from 1 day to 1 year), intermittent hypoxia (range 1-21 h/day, mean 12±5 h/day) and normobaric hypoxia, dataset heterogeneity is a potential

confounder that might affect outcome of the analysis (**Table 2**). We tested for inhomogeneity in with mixed-model regression analysis and found no significant effect of the method (P=0.94), normobaric versus hypobaric hypoxia (P=0.68), continuous versus intermittent hypoxia (P=0.14), or exercise versus no exercise (P=0.23). Thus, because of the absence of significant effect, for the main analysis all data was included.

[Paragraph Number 10.]

Effect of exposure altitude and duration

For clarity of presentation, data was divided into both altitude and duration quartiles (see **Fig. 2**). The random effects model yielded significant main effects for both altitude and duration (both p<0.0001, **Fig. 1**), however, there was also a significant interaction effect (p<0.0001) and therefore the main effects (p-values indicated) should be interpreted with caution. It appears that exposure time must exceed at least 2 weeks at an altitude of more than 4,000 m to exert a significant effect. At lower altitudes, even longer exposure times are needed with altitudes below 3,000 m not yielding a statistically significant result within 4 weeks.

We subsequently performed Monte-Carlo simulation to estimate the required exposure time for an increase in RCV at different altitudes. We entered mean pre and post exposure RCV values with standard deviations along with exposure time into a custom written Matlab (MathWorks, Natick, MA) procedure. From these distributions we randomly drew paired numbers and calculated change in RCV as a function of time. The results of the simulation are presented in **Table 3**. There is an accelerating effect of increasing altitude so that, e.g. reaching a 10% increase at 2,000 m will take 42 to 145 days while at 3,500 m this can be achieved between 23 and 51 days.

[Paragraph Number 11.]

Effect of initial red cell volume

Finally, we examined the effect of the initial red cell volume on the RCV response to hypoxic exposure. To obtain a homologous dataset we restricted the analysis to the special case of exposure between 3,000 and 3,500 m and more than 7 days of stay. The random-effects model showed a highly significant effect of initial RCV with a high initial RCV showing less increase compared to low initial RCV (P<0.0001, **Fig. 3**).

[Paragraph Number 12.]

Discussion

The main findings of the meta-analysis and Monte Carlo simulations are that altitude exposure must exceed 2 weeks at an altitude of > 4,000 m to exert a significant effect on RCV. At lower altitudes, even longer exposure is required with altitudes below 3,000 m not yielding a statistically significant result within 4 weeks. Thus we established an altitude dose-response curve indicating that the hypoxia-induced response in RCV may be slower than widely expected [26].

[Paragraph Number 13.]

Variation in RCV response

We find that the average RCV increase per week for our data is 49±240ml/week which, on average, is in accordance with previous findings with EPO administration [2]. While we find the large variation surprising, similar results may be found in the plasma Epo response to altitude. Ge and co-workers reported that the increase in plasma Epo after 24 hours at 2800 m varied markedly among individuals (n=48), ranging from -41 to 400% which seemed mainly governed by upstream factors related to renal parenchymal PO_2, although genetic factors cannot be ruled out. Although variations in both RCV and the Epo response to altitude are observed these do not seem to be interlinked. In a retrospectively analysis of the Levine data,

Kommentar [PR1]: Carsten, kan du skrive et eller andet om variation i EPO respons a la:
We note the Chapman paper (1998) that retrospectively analyse the Levine data, indicate no correlation between EPO response and erythropoietic response and points to inter-individual variation in responsiveness

Kommentar [CL2]: J Appl Physiol. 2002 Jun;92(6):2361-7

Formatiert: Schriftart: 12 Pt.

Formatiert: Schriftart: 12 Pt.

Formatiert: Schriftart: Times New Roman, 12 Pt.

Formatiert: Schriftart: Times New Roman, 12 Pt.

Formatiert: Schriftart: (Standard) Times New Roman, 12 Pt.

Formatiert: Schriftart: (Standard) Times New Roman, 12 Pt., Nicht Großbuchstaben

Formatiert: Schriftart: (Standard) Times New Roman, 12 Pt., Nicht Hochgestellt/ Tiefgestellt, Nicht Großbuchstaben

Kommentar [CL3]: 1.Ou LC,
2.Salceda S,
3.Schuster SJ,
4.Dunnack LM,
5.Brink-Johnsen T,
6.Chen J,
7.Leiter JC.
(1998) Polycythemic responses to hypoxia: molecular and genetic mechanisms of chronic mountain sickness. J Appl Physiol 84:1242-1251

Formatiert: Schriftart: (Standard) Times New Roman, 12 Pt., Nicht Hochgestellt/ Tiefgestellt, Nicht Großbuchstaben

Formatiert: Schriftart: Times New Roman, 12 Pt.

Formatiert: Schriftart: (Standard) Times New Roman, Nicht Hochgestellt/ Tiefgestellt

Kommentar [CL4]: "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance.
Levine BD, Stray-Gundersen J.
J Appl Physiol. **1997** Jul;83(1):102-12

Formatiert: Schriftart: Times New Roman, 12 Pt., Nicht Fett

Formatiert: Schriftart: Times New Roman, 12 Pt.

Formatiert: Schriftart: Times New Roman, 12 Pt., Nicht Fett

no correlation between the EPO response and erythropoietic response was observed and points to inter-individual variation in responsiveness.

~~our~~ Our finding that >35% of the reported data corresponds to RCV increases per week in excess of 50ml/week is in accordance with Sawka et al [22] which also states that in altitude studies RCV often increase with more than by 50 ml/week. It is intriguing that, in some studies, an increase in RCV is observed within days of exposure to an altitude that did not stimulate polycythemia even after weeks in other studies (**Fig. 1**). This suggests a substantial inter-individual variation [23]. Other candidates that could lead to variation include the use of different measurement techniques or of the application of continuous versus intermittent or normobaric versus hypobaric hypoxia (**Table 2**). Although there was no systematic effect observed when these parameters were entered as ~~a~~ factors, the inclusion of results obtained with different methods may have increased the variance and reduced the power of the analysis. Some evidence suggests slightly different physiological responses to hypobaric vs normobaric hypoxia in regards to ventilation and acute mountain sickness [15,18]. This does, however, not seem to be the case for the RCV response, which is in line with the observation that the Epo response to hypobaric and normobaric hypoxia is also similar [6].

[Paragraph Number 14.]

Effect of initial RCV

One factor that had an effect on the response to altitude exposure was the initial RCV. In this context, we recently failed to observe an increase in RCV after 16 hours/day at 3,000 m for 4 weeks [23]. From the analysis we would expect an 5-10% increase in RCV and we speculated [21] that the absence of an increase in RCV could be related to high initial hemoglobin mass of the included elite athletes that was at the upper end of physiological range (**Table 1**). The present meta-analysis supports this approach (**Fig. 3**). Whereas it is intuitively straightforward that an

Kommentar [CL5]: Individual variation in response to altitude training. Chapman RF, Stray-Gundersen J, **Levine BD**

Formatiert: Schriftart: Times New Roman, 12 Pt.

Formatiert: Schriftart: Times New Roman, 12 Pt., Nicht Fett

Formatiert: Schriftart: Times New Roman, 12 Pt.

Formatiert: Englisch (Großbritannien)

Formatiert: Schriftart: 11 Pt., Englisch (Großbritannien)

Kommentar [L6]: Det her kunne man vel slette.

Formatiert: Schriftart: 11 Pt., Englisch (Großbritannien)

upper limit to RCV expansion must exist, the physiological mechanism governing such a limit is not as easily ~~straightforward~~explained. Although the current meta-analysis indicates that a high initial RCV limits a further expansion at altitude, the study by Kapoor et al. [13] points out that despite a high baseline level, an increase in RCV can still occur if duration and altitude are sufficient (**Fig. 1**). Thus, to what extent a physiological upper limit to RCV exists is not clear from this meta-analysis. However, 17.5 g of hemoglobin per kg bodyweight has been reported in World and Olympic Champion cross country skiers [12], which is markedly higher than values reported in healthy but un-trained high altitude Peruvians [7]. Thus, the upper limit is likely not reached by altitude exposure alone. It should thus be considered that a RCV ceiling may not relate to increased oxygen carrying capacity of blood alone, but could also be related to an upper limit in blood volume and to blood pressure regulation.

[Paragraph Number 15.]

Another explanation for the finding that a high initial RCV attenuates the further increase with hypoxic exposure could be the statistical phenomenon of regression toward the mean. Regression toward the mean may occur when RCV is high on its first measurement, because it will by chance tend to be closer to the average on a second measurement. To avoid drawing wrong conclusions from this meta-analysis (particular **Fig. 3**), the possibility of regression toward the mean should be considered. However, since regression toward the mean is not based on cause and effect, but rather on random error in a natural distribution around a mean, we cannot exclude any underlying physiological mechanisms influencing the effect of initial RCV on erythropoietic response to altitude exposure.

[Paragraph Number 16.]

Is altitude exposure relevant for athletes?

In order to increase RCV and thereby also sea level exercise performance, it is recommended that athletes reside in normobaric or hypobaric hypoxia corresponding to an altitude of 2,500 - 3,000 m for a minimum of 14 hours/day for 3 weeks [26]. The effect of the initial RCV (**Fig. 3**) and the considerable time required to establish a RCV response to medium altitude exposure (**Table 3**) is of relevance for athletes considering engaging into altitude training. Assuming that anecdotal reports of athletes coping bad with altitudes above 3,000 m (increased recovery period after exercise, poor quality of sleep etc.) are correct, then altitudes above this threshold should be avoided. Since only studies in athletes which have demonstrated > 5% increase in hemoglobin mass following altitude training generally report an increase in exercise performance [21], it is recommended that athletes should spend sufficient time at altitude to achieve a corresponding increase in hemoglobin mass. A gain in hemoglobin mass of 7.5% requires between 35 and 56 days of live-high train-low or 25 to 47 days of continuous exposure at 2,500 m to achieve a > 95 % probability for an increase (**Table 3**). This is further extended by a high initial RCV which results in lower probability for a further increase to occur. This questions the feasibility of altitude training for elite athletes, and the potential gains by altitude training, at least in athletes with a high RCV starting point, should therefore be carefully reconsidered.

[Paragraph Number 17.]

Publication bias

We found some indications for publication bias in the tendency for studies with small changes and large variations to be missing from the analysis. Thus, we cannot exclude that the analysis over-estimates the positive outcome. On the other hand, almost 40% of the included data points reported negative findings and 50% of the data points reported less than 2.5% increase.

[Paragraph Number 18.]

Dataset heterogeneity

Roughly a fourth of the data originates from studies involving intermittent hypoxia (**Table 2**). We were not able to detect any significant differences when the two hypoxic exposures were compared in the random-effects model. On the other hand, Monte-Carlo analysis revealed that when the data obtained from intermittent hypoxic exposure was removed from the analysis, time to a 95% chance for an increase in RCV generally decreased with 4-7 days, particularly in around 3,000 m above sea-level where most of the intermittent hypoxia studies were performed. We recorded iron supplementation and in 72% of the studies no iron supplementation was performed. Levine & Stray-Gundersen [14] suggested that iron supplementation is needed only if iron stores are low. Parisotto et al. [16] could not find an effect of iron supplementation when Epo was administrated. Thus, iron availability may not be an issue for healthy individuals. However, it is still not straightforward to access the impact of iron supplementation as e.g. a majority of the studies with iron supplementation use intermittent hypoxia. Thus, there is a risk of co-variance between the parameters. This extends to possible differences between normobaric and hypobaric hypoxia, gender and the methods used. In general, we saw no indications that normobaric hypoxia was producing a different response than hypobaric hypoxia which is in accordance with our recent findings [17]. Likewise we were not able to detect any differences between the few studies reporting only women and those with only men or a mixed population. What we note, however, is that to our knowledge no studies have looked specifically at gender differences in RCV response to altitude dwelling and very few studies report males and females separately. Finally, the method to estimate RCV may also introduce bias. According to Sawka el al. [22], CO re-breathing may overestimate not only the RCV but also changes in RCV after altitude exposure because of CO loss to myoglobin or other ironporphyrin molecules (i.e. in the liver) and according changes in the quantity of these molecules following altitude exposure. However, Gore et al. [9] and Thomsen et al. [24] concluded that the two measurement were equivalent. Furthermore, CO

loss to myoglobin is assumed minimal (1.6 ml over 10 min[8]), ~~so~~and even if myoglobin should change as a consequence of altitude exposure the effect hereof on RCV must be assumed ~~to be very minimal~~neglectable. Finally, it is unknown if these extra-vascular iron molecules actually increase with hypoxic exposure.

Kommentar [L7]: Ikke malt direkte.

Kommentar [L8]: Kan vel slettes nu?

[Paragraph Number 19.]

Conclusion

The physiological process of red cell expansion occurs relatively slowly with only minor chances for an increase to occur within 2 weeks and exposure times longer than 4 weeks generally required. Also the accepted paradigm that altitudes < 3,000 m are sufficient to trigger a robust RCV should be reconsidered particularly if the initial RCV is high. This, in combination with a dependency on initial red cell volume, suggests that e.g. athletes may need to spend more time at altitude to see an effect on red cell volume than commonly recommended

[Paragraph Number 20.]

Author contributions

CL initiated the investigation; PR and VD designed the literature search; CS and VD extracted the data; PR performed the analysis. All authors wrote the paper, approved the final version and declare no conflicts of interest.

[Paragraph Number 21.]

Acknowledgements

We thank Stefan L. Mabit for invaluable comments on the statistical analysis. This study was supported by a grant from the Danish National Research Council (10-094110) and the Zurich

Center of Integrative Human Physiology. The results of the present study do not constitute endorsement by ACSM.

[Paragraph Number 22.]

References

1. Ashenden MJ, Gore CJ, Martin DT, Dobson GP, Hahn AG. Effects of a 12-day "live high, train low" camp on reticulocyte production and haemoglobin mass in elite female road cyclists. *Eur J Appl Physiol*. 1999;80:472-8.

2. Berglund B, Ekblom B. Effect of recombinant human erythropoietin treatment on blood pressure and some haematological parameters in healthy men. *J Intern Med*. 1991;229(2):125-30.

3. Berglund B, Gennser M, ÖRnhagen H, ÖStberg C, Wide L. Erythropoietin concentrations during 10 days of normobaric hypoxia under controlled environmental circumstances. *Acta Physiologica Scandinavica*. 2002;174(3):225-9. 10.1046/j.1365-201x.2002.00940.x.

4. Curtin F, Altman DG, Elbourne D. Meta-analysis combining parallel and cross-over clinical trials. I: Continuous outcomes. *Statistics in Medicine*. 2002;21(15):2131-44. 10.1002/sim.1205.

5. Eckardt KU, Boutellier U, Kurtz A, Schopen M, Koller EA, Bauer C. Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. *Journal of Applied Physiology*. 1989;66(4):1785-8.

6. Fandrey J. Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression. *Am J Physiol Regul Integr Comp Physiol*. 2004;286(6):R977-88. Prepublished on 2004/05/15 as DOI 10.1152/ajpregu.00577.2003

286/6/R977 [pii].

7. Gamboa A, Gamboa JL, Holmes C, et al. Plasma catecholamines and blood volume in native Andeans during hypoxia and normoxia. *Clin Auton Res*. 2006;16(1):40-5. Prepublished on 2006/02/16 as DOI 10.1007/s10286-006-0305-z.

8. Garvican LA, Burge CM, Cox AJ, Clark SA, Martin DT, Gore CJ. Carbon monoxide uptake kinetics of arterial, venous and capillary blood during CO rebreathing. *Experimental physiology*. 2010;95(12):1156-66. Prepublished on 2010/09/08 as DOI 10.1113/expphysiol.2010.054031.

9. Gore CJ, Hopkins WG, Burge CM. Errors of measurement for blood volume parameters: a meta-analysis. *Journal of Applied Physiology*. 2005;99(5):1745-58. Prepublished on 2005/06/25 as DOI 10.1152/japplphysiol.00505.2005.

10. Hedges LV, Gurevitch J, Curtis PS. The Meta-Analysis of Response Ratios in Experimental Ecology. *Ecology*. 1999;80(4):1150-6.

11. Jelkmann W. Erythropoietin after a century of research: younger than ever. *Eur J Haematol*. 2007;78(3):183-205.

12. Jelkmann W. Regulation of erythropoietin production. *J Physiol*. 2011;589(Pt 6):1251-8. Prepublished on 2010/11/17 as DOI 10.1113/jphysiol.2010.195057.

13. Kapoor SC, Chatterjee AK. Hematological response among new arrival at high-altitude. *Indian J Med Res*. 1978;67(MAR):428-34.

14. Levine BD, Stray-Gundersen J. A practical approach to altitude training: where to live and train for optimal performance enhancement. *International journal of sports medicine*. 1992;13 Suppl 1:S209-12. Prepublished on 1992/10/01 as DOI 10.1055/s-2007-1024642.

15. Loeppky JA, Icenogle M, Scotto P, Robergs R, Hinghofer-Szalkay H, Roach RC. Ventilation during simulated altitude, normobaric hypoxia and normoxic hypobaria. *Respir Physiol*. 1997;107(3):231-9. Prepublished on 1997/03/01 as DOI S0034568797025231 [pii].

16. Parisotto R, Wu M, Ashenden MJ, et al. Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. *Haematologica*. 2001;86(2):128-37. Prepublished on 2001/02/27 as DOI.

17. Rasmussen P, Nordsborg N, Taudorf S, et al. Brain and skin do not contribute to the systemic rise in erythropoietin during acute hypoxia in humans. *FASEB J*. 2012;26(5):1831-4. Prepublished on 2012/02/11 as DOI fj.11-191692 [pii]

10.1096/fj.11-191692.

18. Roach RC, Loeppky JA, Icenogle MV. Acute mountain sickness: increased severity during simulated altitude compared with normobaric hypoxia. *J Appl Physiol*. 1996;81(5):1908-10. Prepublished on 1996/11/01 as DOI.

19. Robach P, Cairo G, Gelfi C, et al. Strong iron demand during hypoxia-induced erythropoiesis is associated with down-regulation of iron-related proteins and myoglobin in human skeletal muscle. *Blood*. 2007;109(11):4724-31. 10.1182/blood-2006-08-040006.

20. Robach P, Dechaux M, Jarrot S, et al. Operation Everest III: role of plasma volume expansion on VO(2)(max) during prolonged high-altitude exposure. *Journal of Applied Physiology*. 2000;89(1):29-37. Prepublished on 2000/07/25 as DOI.

21. Robach P, Lundby C. Is Live High - Train Low altitude training relevant for elite athletes with already high total hemoglobin mass? *Scand J Med Sci Sports*. 2012;accepted for publication.

22. Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Med Sci Sports Exerc*. 2000;32(2):332-48. Prepublished on 2000/02/29 as DOI.

23. Siebenmann C, Robach P, Jacobs RA, et al. "Live high-train low" using normobaric hypoxia: a double-blinded, placebo-controlled study. *J Appl Physiol*. 2012;112(1):106-17. Prepublished on 2011/10/29 as DOI japplphysiol.00388.2011 [pii]

10.1152/japplphysiol.00388.2011.

24. Thomsen JK, Fogh-Andersen N, Bulow K, Devantier A. Blood and plasma volumes determined by carbon monoxide gas, 99mTc-labelled erythrocytes, 125I-albumin and the T 1824 technique. *Scandinavian journal of clinical and laboratory investigation*. 1991;51(2):185-90. Prepublished on 1991/04/01 as DOI.

25. Weil JV, Jamieson G, Brown DW, Grover RF. The red cell mass-arterial oxygen relationship in normal man: Application to patients with chronic obstructive airway disease. *J Clin Invest*. 1968;47(7):1627-39.

26. Wilber RL, Stray-Gundersen J, Levine BD. Effect of Hypoxic "Dose" on Physiological Responses and Sea-Level Performance. *Med Sci Sports Excerc*. 2007;39(9):1590-9.

Supplemental Digital Content

File1: Schematic view of the literature review process with a list of the papers retrieved.

Figure legends

Figure 1. Red blood cell response to exposure time and altitude. Bubble size are scaled to log response ratio and weighted according to study sample size but to preserve presentation fidelity transformed with square root function. Filled bubbles indicate increased red cell increase while open bubbles indicate decreases.

Figure 2. Red blood cell response. Data is presented in altitude and exposure quartiles. Note that there was a significant interaction between the effect of attitude and exposure duration ($p<0.0001$) and therefore the main effects (p-values indicated) should be interpreted with caution. It is, however, clear that exposure time must exceed 17 days at an altitude of more than 4,000 m to exert a significant effect on the oxygen carrying capacity of the blood. Data are weighted log response ratios with error bars indicating 95% confidence intervals.

Figure 4: The effect of initial hemoglobin mass on response to hypoxic exposure. To ensure clarity of presentation and to avoid confounding bias, data is limited to exposure times between 7 and 40 days at altitudes between 3,000 and 3,500 m. On the abscissae log response ratios with 95% confidence intervals are presented and on the ordinate hemoglobin mass with standard deviations. Mean values for altitude (3,327, 3,097, 3,070 m) and exposure (21, 19, 23 days) for the three hemoglobin mass groups (N=13, 14 and 15) are not different.